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# REGULATION OF ELASTASE AND PLASMINOGEN ACTIVATOR SECRETION IN RESIDENT AND INFLAMMATORY MACROPHAGES BY RECEPTORS FOR THE Fc DOMAIN OF IMMUNOGLOBULIN G

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The macrophage is a versatile cell that, in a normal environment, resides in an unstimulated state. Upon encountering inflammatory or immunologic stimuli (1-4), it alters its phenotype and becomes stimulated or activated<sup>1</sup> for secretory, microbicidal, or tumoricidal activities. The precise molecular steps leading to stimulation or activation are not known, although they may be initiated by the interaction of specific receptors on macrophages with ligands such as lymphokines or endotoxin, or by the direct interaction of macrophages with other cells.

The response of macrophages to specific ligands can be compared to the cellular response to hormones. The interaction of a hormone with a specific cellular receptor may provoke a variety of responses by the cell (5). These may be early, occurring within seconds to minutes of exposure, or late, occurring after days of continuous exposure to hormones (6). Early responses are the direct result of hormone-receptor interaction, whereas late effects most likely require the generation of cytoplasmic messengers. Specific receptors on macrophages include receptors for the Fc domain of various immunoglobulin G subclasses (7, 8) and for the fragments of the third component of complement (C3) (9) that stimulate phagocytosis. Many of the early responses to receptor triggering in macrophages have been described, including triggering of the respiratory burst and arachidonate metabolism; however, the late responses have not been studied in detail.

Macrophages secrete more than 50 substances, including neutral proteinases (10). Neutral proteinases are secreted in increased amounts during stimulation of macrophages, and have been used as phenotypic markers for macrophage activation (10-14). Phagocytosis of particles has been implicated as an event that leads to the enhanced secretion of neutral proteinases, and the nondigestibility

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<sup>1</sup> "Stimulated" macrophages are macrophages that encounter inflammatory stimuli and are high in secretory activity but do not have microbicidal or tumoricidal activity. "Activated" macrophages are macrophages that have encountered immunologic stimuli and acquired microbicidal or tumoricidal activity.

of particles or the ability of particles to induce hexose monophosphate shunt may be responsible for the stimulation itself (11–14). In this study, we have shown that the interaction of ligands with macrophage phagocytic receptors regulates the secretion of the neutral proteinases, elastase and plasminogen activator.

### Materials and Methods

**Materials.** Dulbecco's modified Eagle's medium high glucose formulation (4.5 g glucose/l) (DME)<sup>2</sup> was obtained from the Tissue Culture Facility, University of California, San Francisco, CA. Fetal bovine serum (FBS), penicillin-streptomycin solution, lactalbumin hydrolysate (LH), and Hanks' balanced salt solution (HBSS) were purchased from Grand Island Biological Co. (Grand Island, NY). Cytochalasin B and uniform-size latex particles were purchased from Aldrich Chemical Co. (Milwaukee, WI) and Dow Chemical Co. (Indianapolis, IN), respectively. The Fc fragment of rabbit IgG, which was purchased from Cappel Laboratories, Inc. (West Chester, PA), separated as a single band of apparent  $M_r$  30,000 under reducing conditions in sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

**Cell Culture.** Resident or inflammatory mouse peritoneal macrophages were harvested from female Swiss Webster mice (CD-1; Charles River Breeding Laboratories, Inc., Wilmington, MA) as described previously (15). To obtain inflammatory macrophages mice were injected intraperitoneally with either 1 ml of 3% aged Brewer's thioglycollate broth or 0.5 ml of 5 mM NaIO<sub>4</sub> in 0.15 M NaCl 4 d before harvest (16). Cells were plated in DME supplemented with 50 U/ml penicillin G, 50 µg/ml streptomycin, and 10% heat-inactivated FBS (DME-10% FBS) in 16-mm-diameter multiwell plates (Costar, Cambridge, MA) either directly or on 12-mm coverglasses placed in the plates. Cells were allowed to adhere for 2–4 h, were washed vigorously with HBSS, and were incubated in DME-10% FBS for 24 h. Cells were then washed with HBSS and incubated with various particles in DME-10% FBS or in serum-free DME supplemented with penicillin-streptomycin solution and 0.2% LH (DME-LH). After 2–26 h, particles were washed off and the culture was continued in DME-LH with changes of medium every 24–72 h. The conditioned medium was used for enzyme assays.

**Elastase Assay.** Macrophage elastase activity in conditioned medium was assayed as described previously (15) by using as substrate [<sup>3</sup>H]NaBH<sub>4</sub>-reduced elastin from bovine ligamentum nuchae in the presence of sodium dodecyl sulfate. One unit of elastase activity is defined as the activity to solubilize 1 µg of elastin/h at 37°C.

**Plasminogen Activator Assay.** Plasminogen activator activity in conditioned medium or in living macrophages was assayed by using plates coated with <sup>125</sup>I-labeled bovine fibrin as described previously (11, 17). Briefly, plasminogen activator activity in conditioned medium was measured by monitoring the release of labeled peptides in the presence of 6 nM purified bovine plasminogen in 50 mM Tris/HCl, pH 7.8, at 37°C; 50 µl of conditioned medium was assayed in a final reaction volume of 0.5 ml/well. One unit of plasminogen activator is defined as the activity to degrade 5% (~1 µg) of substrate/h at 37°C. The activity of plasminogen activator in intact cells was assayed by plating cells directly on <sup>125</sup>I-labeled fibrin plates in DME-10% FBS and then monitoring the release of labeled peptides in the presence of plasminogen in DME-LH at 37°C. Fibrinolysis in the absence of plasminogen in DME-10% FBS or DME-LH was <5% per 24 h. The two assays both detect plasminogen activator in macrophages, but there may be some differences because some of the plasminogen activator in macrophages has been shown to be membrane-bound (18, 19). The conditioned medium assay is likely to detect secreted

<sup>2</sup> Abbreviations used in this paper: DME, Dulbecco's modified Eagle's medium; EIgG, sheep erythrocytes coated with IgG fraction of rabbit anti-erythrocyte antibody; EIgGC, sheep erythrocytes coated with IgG fraction of rabbit anti-erythrocyte antibody plus mouse complement; EIgMC, sheep erythrocytes coated with IgM fraction of rabbit anti-erythrocyte antibody plus mouse complement; FBS, fetal bovine serum; HBSS, Hanks' balanced salt solution; LH, lactalbumin hydrolysate.

plasminogen activator only, and the intact cell assay is likely to detect membrane-bound as well as secreted plasminogen activator.

*Preparation of Sheep Erythrocytes.* Sheep erythrocytes coated with IgG fraction of rabbit anti-erythrocyte antibody (EIgG), IgM fraction of rabbit anti-erythrocyte antibody plus mouse complement (EIgMC), or IgG fraction of rabbit anti-erythrocyte antibody plus mouse complement (EIgGC) were prepared essentially as described by Bianco (20). To prepare EIgG, 0.1–1 ml of  $2 \times 10^9$ /ml sheep erythrocytes in HBSS was incubated with the same volume of 20–50  $\mu$ g/ml or other concentrations of the IgG fraction of rabbit anti-sheep erythrocyte antibody (Cordis Laboratories, Miami, FL; lot nos. 60625 and R4110) for 30 min at 37°C. After washing, EIgG were suspended in DME-LH at  $2 \times 10^8$ /ml.

Sheep erythrocytes coated with mouse monoclonal anti-sheep erythrocyte IgG of various subclasses were prepared in the same manner. The monoclonal anti-sheep erythrocyte IgG<sub>2a</sub> (UN-2) was the gift of Dr. B. Diamond (Albert Einstein Medical School, Bronx, NY), and IgG fraction was purified from ascites by affinity chromatography on protein A-Sepharose (Pharmacia Fine Chemicals, Piscataway, NJ) (21). The monoclonal anti-sheep erythrocyte IgG<sub>2b</sub> (Sp 2) was obtained from Accurate Chemical and Scientific Corporation (Westburg, NY), and the ascites was used without purification. The hybridoma cell line producing the monoclonal anti-sheep erythrocyte IgG<sub>3</sub> (N-S.7) was obtained from the Salk Institute (San Diego, CA), and the IgG fraction was purified from the culture supernatant by chromatography on protein A-Sepharose.

To prepare EIgMC,  $2 \times 10^9$ /ml of sheep erythrocytes in HBSS were first incubated with the same volume of 7  $\mu$ g/ml of the IgM fraction of rabbit anti-erythrocyte antibody (Cordis Laboratories, Miami, FL) for 30 min at 37°C to obtain EIgM. After washing, EIgM were suspended at  $1 \times 10^9$ /ml in veronal-buffered saline with glucose and incubated with the same volume of a 1:5 dilution of serum from C5-deficient AKR mice in veronal-buffered saline with glucose containing 1 mM phenylmethylsulfonylfluoride for 10 min at 37°C. After washing, EIgMC were suspended in DME-LH at  $2 \times 10^8$ /ml. EIgMC prepared in this way are most likely to be recognized by macrophages by the complement receptor I, CR I (9).

To prepare EIgGC, EIgG prepared as described were coated with AKR mouse serum in the same manner as EIgMC.

*Rosette Formation and Phagocytosis.* To evaluate rosette formation, macrophages were incubated with EIgG or EIgMC for 60 min at 37°C and fixed in 1.25% glutaraldehyde in phosphate-buffered saline, and erythrocytes that were attached to or associated with macrophages were counted under a phase contrast microscope (20). To evaluate phagocytosis, erythrocytes associated with macrophages were counted after hypotonic treatment with 1:5 diluted phosphate-buffered saline, which lyses erythrocytes attached to the outside of macrophages. Attachment or association of three or more erythrocytes signified rosette formation or phagocytosis. In some experiments, rosette formation or phagocytosis was expressed as an index of the number of erythrocytes bound or phagocytosed per 100 macrophages.

*Determination of the Number of IgG Bound to Erythrocytes.* To determine the number of IgG bound to erythrocytes, rabbit anti-erythrocyte antibody was radiolabeled with <sup>125</sup>I by using Iodogen (Pierce Chemical Co., Rockford, IL) (22). Erythrocytes (0.1 ml of  $2 \times 10^9$ /ml) were incubated with 0.1 ml of various concentrations of <sup>125</sup>I-labeled rabbit anti-erythrocyte antibody for 30 min at 37°C, and the bound IgG was determined by counting in a gamma counter.

## Results

*EIgG Stimulates Elastase and Plasminogen Activator Secretion by Resident Macrophages.* Mouse resident peritoneal macrophages bind and ingest EIgG via their Fc receptors (23). Within 60 min of incubation of macrophages with EIgG prepared using a subagglutinating titer of rabbit anti-erythrocyte antibody, >90% of macrophages had bound EIgG. When resident macrophages were incubated

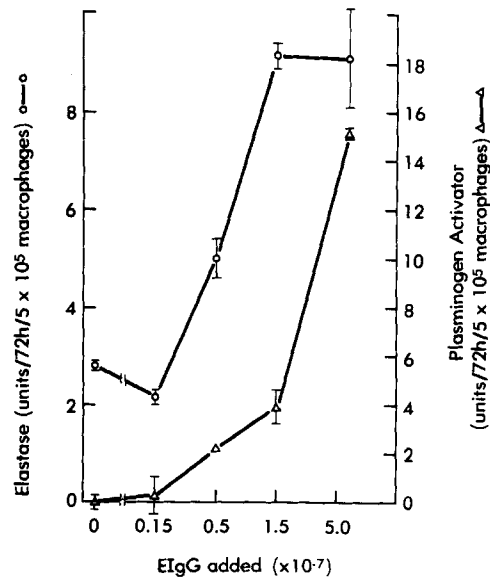


FIGURE 1. Dose-dependent stimulation by EIgG of neutral proteinase secretion by resident macrophages. Macrophages ( $5 \times 10^5$ ) were incubated with various numbers of EIgG in DME-10% FBS for 24 h. The macrophages were then washed and incubated in DME-LH for 72 h to obtain conditioned medium, which was assayed for elastase and plasminogen activator activity.

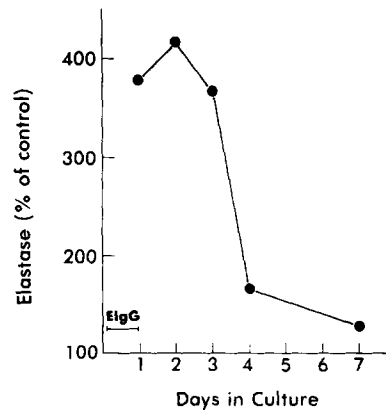


FIGURE 2. Duration of stimulation by EIgG of elastase secretion by resident macrophages. Macrophages ( $5 \times 10^5$ ) were incubated with EIgG ( $5 \times 10^7$ ) for 0–24 h and the conditioned medium was collected and replaced with fresh DME-LH daily. The conditioned medium was assayed for elastase activity, and the results are expressed as percentage of elastase secreted by macrophages incubated without EIgG. The control rate of elastase secretion gradually increased with time from 0.5 unit/24 h/ $5 \times 10^5$  macrophages at day 1 to 1.0 unit/24 h/ $5 \times 10^5$  macrophages at day 7.

with EIgG continuously for 24 h, washed, and then incubated in serum-free medium, their secretion of elastase was increased two- to sixfold and their secretion of plasminogen activator was increased >20-fold compared to control cells (Fig. 1). The stimulated secretion of elastase by macrophages was sustained for up to 3 d (Fig. 2); thereafter, the secretion rate was reduced to the control

TABLE I  
Duration of Elastase Secretion by Resident Macrophages Stimulated by  
EIgG or Latex

Time after phago- cytosis	Elastase secreted		
	Control	EIgG	Latex
<i>d</i>	<i>U/72 h/5 × 10<sup>5</sup> macrophages</i>		
1-4	0.80 ± 0.08	2.08 ± 0.43	1.49 ± 0.02
4-7	2.23 ± 0.99	1.63 ± 0.29	5.56 ± 0.64
7-10	1.62 ± 0.26	2.40 ± 0.63	4.69 ± 0.18
10-13	3.91 ± 0.01	4.83 ± 0.17	3.61 ± 1.08

Macrophages ( $5 \times 10^5$ ) were incubated with EIgG ( $5 \times 10^7$ ) or latex (25  $\mu$ g, polyvinyltoluene, 2- $\mu$ m diameter) in DME-LH for 24 h. Control macrophages were incubated with medium alone. After washing, the medium was replaced by fresh DME-LH and collected at 3-d intervals. The conditioned medium was assayed for elastolytic activity.

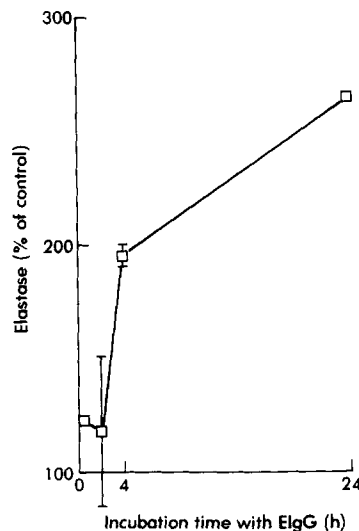


FIGURE 3. Dependence of elastase secretion by resident macrophages on time of incubation with EIgG. Macrophages ( $4.4 \times 10^5$ ) were incubated with EIgG ( $4.4 \times 10^7$ ) in DME-LH. At 0.5, 2, and 4 h, EIgG were washed off and the conditioned medium was saved. Macrophages were further incubated with fresh DME-LH. At 24 h the conditioned medium was removed and pooled with the conditioned medium from an earlier time point. The elastolytic activity secreted during the entire 24-h period (pooled medium, during and after phagocytosis) was determined. Results are expressed as percentage of elastase secretion by macrophages incubated without EIgG.

level. This time course was in contrast to the stimulation of elastase by latex particles, which was sustained for up to 10 d (Table I).

*Stimulation of Secretion of Elastase, But Not Plasminogen Activator, Requires Continued Exposure of Macrophages to EIgG.* Because we had incubated the macrophages with EIgG for 24 h in the experiments just described, we then determined the minimal time of cell-ligand contact required for the stimulated secretion. Incubation of macrophages with EIgG for  $<2$  h resulted in almost no stimulation of elastase secretion (Fig. 3). In contrast, plasminogen activator activity, as detected

TABLE II  
Time Dependence of Plasminogen Activator Secretion in Intact Resident Macrophages Stimulated by EIgG

Time of plasminogen activator assay <i>h</i>	Time of phagocytosis <i>h</i>	Plasminogen activator	
		Control	EIgG
		<i>U/2 h/4.4 × 10<sup>5</sup> macrophages</i>	
2	0-2	0.03 ± 0.03	0.11 ± 0.04
20	0-20	0.20 ± 0.07	1.28 ± 0.17
20	0-2	0.20 ± 0.07	0.84 ± 0.16

Macrophages ( $4.4 \times 10^5$ ) were plated on  $^{125}\text{I}$ -fibrin and cultivated for 24 h in DME-10% FBS. The plates were washed and incubated with EIgG ( $4.4 \times 10^7$ ) in DME-LH for 2 h or 20 h. Control macrophages were incubated with medium alone. After washing, 6 nM bovine plasminogen activator was added and  $^{125}\text{I}$ -fibrin degradation during 2 h was monitored.

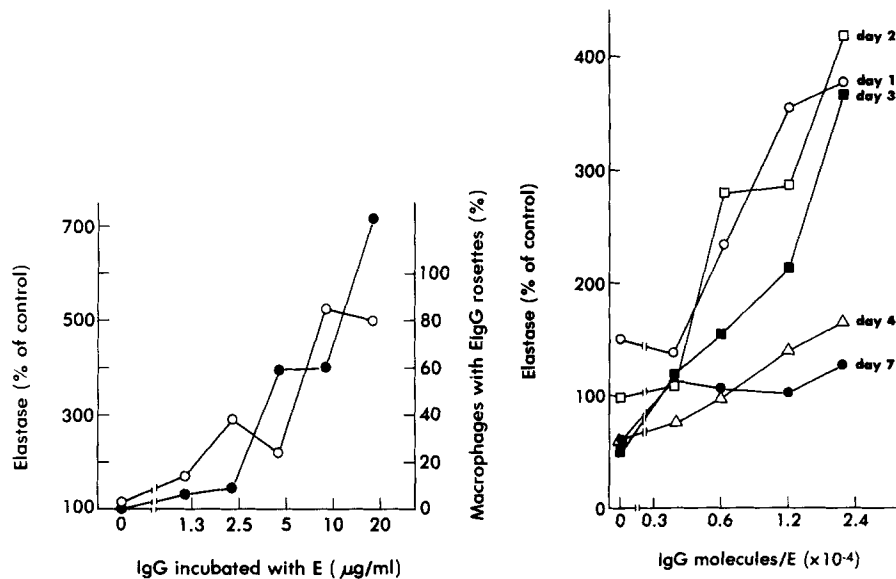


FIGURE 4. Dependence of elastase secretion by resident macrophages on titer of IgG bound to erythrocytes. (a) Sheep erythrocytes (E) were incubated with anti-erythrocyte IgG (lot no. R4110) at indicated concentrations. EIgG ( $5 \times 10^7$ ) thus prepared were incubated in DME-LH with macrophages ( $5 \times 10^5$ ) for 24 h. The macrophages were then washed and incubated in DME-LH for 48 h to obtain the conditioned medium. The conditioned medium was assayed for elastase activity, and the results are expressed as percentage of elastase secretion by macrophages incubated without EIgG. The percentage of macrophages forming rosettes with 3 or more EIgG was determined after 60 min incubation. (b) Sheep erythrocytes (E) were incubated with anti-erythrocyte IgG (lot no. 60625) at concentrations of 6.75, 13.5, 27, and 54  $\mu\text{g/ml}$ , which resulted in erythrocytes coated with  $0.37 \times 10^4$ ,  $0.62 \times 10^4$ ,  $1.3 \times 10^4$ , and  $2.1 \times 10^4$  IgG molecules per cell, respectively. Resulting EIgG ( $5 \times 10^7$ ) were incubated with macrophages ( $5 \times 10^5$ ) in DME-LH for 24 h and the conditioned medium was collected and replaced with fresh DME-LH daily. The conditioned medium was assayed for elastase activity, and the results are expressed as percentage of elastase secretion by macrophages incubated without EIgG.

by the intact cell assay, was stimulated after exposure to EIgG for 2 h, although continued exposure for 20 h stimulated even more activity (Table II).

*Stimulation of Elastase Secretion by EIgG Is Dependent on Ligand Density.* When EIgG were prepared by using various concentrations of IgG, more stimulation of elastase was seen with increasing concentrations of IgG (Fig. 4 a). This concentration dependence for stimulation of elastase secretion closely paralleled the increased formation of EIgG rosettes with macrophages. The stimulation of elastase secretion was detectable at  $>6.2 \times 10^3$  molecules of IgG/erythrocyte (Fig. 4 b). Although we sometimes observed a stimulatory effect by erythrocytes alone during 0–24 h of incubation, this effect was never sustained after this period. Soluble Fc fragments of rabbit IgG did not stimulate elastase secretion at  $\leq 1 \mu\text{M}$ .

*Binding of EIgG Without Ingestion Stimulates Elastase Secretion.* Although most of the EIgG incubated with macrophages bind to the cells, a smaller number are ingested, particularly at lower ligand densities (20, 23). To determine whether stimulation of elastase secretion required ingestion or whether it occurred after receptor binding alone, macrophages were incubated with EIgG in the presence of 0.5–4  $\mu\text{g/ml}$  of cytochalasin B, which inhibits ingestion but not binding (24). At 24 h, macrophages incubated with  $>1 \mu\text{g/ml}$  cytochalasin B were smaller, had associated rosettes of EIgG, and did not show the inclusions of ingested erythrocytes typical of untreated cells. At 0.5  $\mu\text{g/ml}$ , the inhibition of phagocytosis was partial. It is interesting to note that in the presence of cytochalasin B most of the EIgG were lysed by the macrophages, perhaps by antibody-dependent cell-mediated cytotoxic mechanisms, and only adherent ghosts remained at 24 h. Although cytochalasin B induced morphologic changes in resident peritoneal macrophages, it did not by itself affect elastase secretion in this series of experiments (Table III). Because phagocytosis was not required for the stimulation of secretion, it is likely that stimulation via the Fc receptor was induced at the level of receptor-ligand interaction.

*All Subclasses of Mouse IgG Stimulate Elastase Secretion.* Mouse macrophages

TABLE III  
Effect of Cytochalasin B on Secretion of Elastase by Resident  
Macrophages Stimulated by EIgG

Treatment	Elastase secreted	
	Control	EIgG
	<i>U/24 h/5 × 10<sup>5</sup> macrophages</i>	
None	0.63 ± 0.01	2.80 ± 0.25
Dimethyl sulfoxide (0.16%)	0.83 ± 0.10	1.93 ± 0.12
Cytochalasin B, 0.5 $\mu\text{g/ml}$	0.95 ± 0.18	2.22 ± 0.15
1.0 $\mu\text{g/ml}$	0.64 ± 0.17	2.86 ± 0.14
2.0 $\mu\text{g/ml}$	0.57 ± 0.03	2.26 ± 0.01
4.0 $\mu\text{g/ml}$	0.50 ± 0.08	2.27 ± 0.05

Macrophages ( $5 \times 10^5$ ) were incubated with EIgG ( $5 \times 10^7$ ) in the presence of cytochalasin B at indicated concentrations in DME-LH for 24 h. Control macrophages were incubated without EIgG. Collected medium was assayed for elastase. Cytochalasin B was dissolved in dimethyl sulfoxide at a final concentration of 0.16% in culture.



have three subclass-specific Fc receptors: one is trypsin-sensitive and specific for monomer and aggregated forms of mouse IgG<sub>2a</sub>. Two others are trypsin-resistant and specific for the aggregated forms of mouse IgG<sub>2b</sub> or IgG<sub>1</sub> and of mouse IgG<sub>3</sub>, respectively (8, 25). The functional differences among these receptors have not been elucidated. Therefore, we examined whether these receptors would stimulate the secretion of elastase differently. We coated sheep erythrocytes with mouse monoclonal anti-erythrocyte antibodies of IgG<sub>2a</sub>, IgG<sub>2b</sub>, and IgG<sub>3</sub> subclasses. Both IgG<sub>2a</sub> and IgG<sub>2b</sub> stimulated elastase secretion (Fig. 5). The stimulation of elastase by IgG<sub>3</sub> was less prominent than that by the other two. Because IgG<sub>3</sub> ligand bound with weak affinity under our conditions, we were unable to determine whether IgG<sub>3</sub>-specific Fc receptor stimulates the secretion of elastase to the same extent as the IgG<sub>2a</sub>- and IgG<sub>2b</sub>-specific receptors.

*Binding of EIgMC Stimulates Elastase Secretion Transiently.* Resident mouse peritoneal macrophages bind but do not ingest EIgMC via the C3b-receptor, in contrast to activated macrophages, which mediate ingestion (26). Because binding of EIgG to the receptor stimulated elastase secretion, it was of interest to examine whether the binding of EIgMC without phagocytosis would stimulate the secretion of elastase. At least 90% of resident peritoneal macrophages bound EIgMC. Mouse macrophages do not have receptors for rabbit IgM (26), and therefore EIgM were not bound.

When macrophages were incubated with EIgMC, in two independent experiments the maximal response of a three- to fourfold increase in elastase secretion compared with erythrocytes or EIgG was seen within 2–4 h of treatment (Fig. 6). However, this secretion rate was not sustained after this initial period and approached that of control cultures by 24 h, so that the overall secretion for the 24-h period was only 1.2- to 2.5-fold over control. When sheep erythrocytes

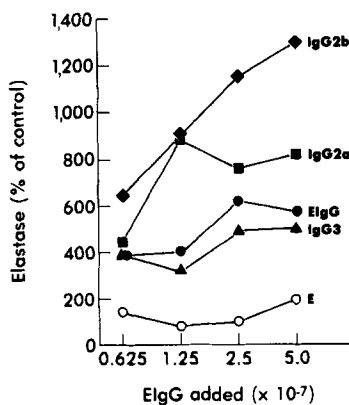


FIGURE 5. Effect of binding of EIgG to subclass-specific Fc receptors on elastase secretion by resident macrophages. Sheep erythrocytes (E) were coated with mouse monoclonal anti-erythrocyte antibody (320  $\mu$ g/ml) of various isotypes to make complexes. Macrophages ( $2 \times 10^5$ ) were incubated with the indicated number of erythrocytes, EIgG, or erythrocytes coated with mouse monoclonal anti-erythrocyte antibody for 24 h in DME-LH. The macrophages were then washed and incubated in DME-LH for 48 h to obtain the conditioned medium. The conditioned medium was assayed for elastase activity, and the results are expressed as percentage of elastase secretion by macrophages incubated without EIgG. The rosette indices of each subclass in this experiment were 876 for IgG<sub>2a</sub>, 856 for IgG<sub>2b</sub>, 231 for IgG<sub>3</sub>, and 915 for rabbit IgG at 60 min.

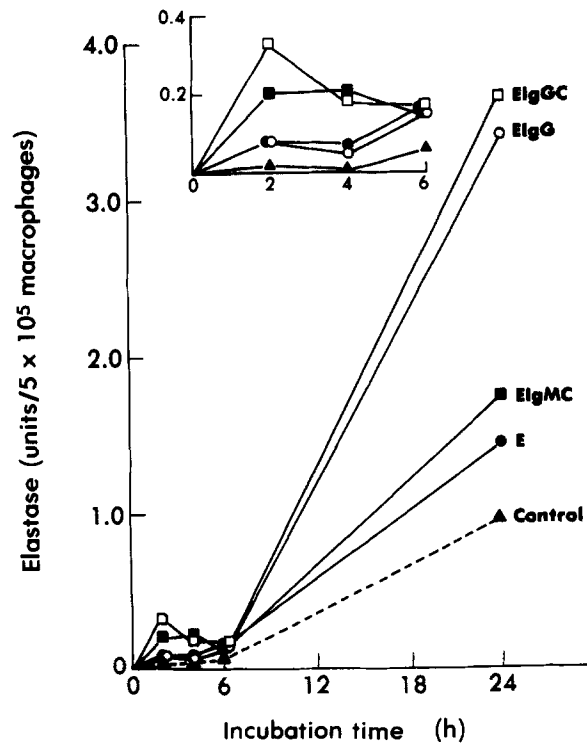


FIGURE 6. Stimulation by EIgMC of elastase secretion by resident macrophages. Macrophages ( $5 \times 10^5$ ) were incubated in DME-LH with  $5 \times 10^7$  erythrocytes (E), EIgG, EIgMC, EIgGC, or without any phagocytic stimuli. After the indicated time of incubation, the conditioned medium was collected and assayed for elastase activity.

TABLE IV  
Effect of EIgG on Neutral Proteinase Secretion by Inflammatory Macrophages

Macrophage type	Elastase secreted		Plasminogen activator secreted	
	Control	EIgG	Control	EIgG
	<i>U/75 h/5 × 10<sup>5</sup> macrophages</i>		<i>U/75 h/5 × 10<sup>5</sup> macrophages</i>	
Resident	2.8 ± 0.0	8.6 ± 1.0	0	15.1 ± 0.2
Thioglycollate-elicited	10.0 ± 0.8	11.7 ± 1.0	21.0 ± 1.1	15.0 ± 2.6
Periodate-elicited	9.2 ± 1.6	11.8 ± 0.7	2.3 ± 1.1	20.5 ± 1.1

Resident, thioglycollate-elicited, or periodate-elicited macrophages ( $5 \times 10^5$ ) were incubated with EIgG ( $5 \times 10^7$ ) for 24 h in DME-10% FBS. Control macrophages were incubated without EIgG. The macrophages were then washed and incubated in DME-LH for 72 h to obtain conditioned medium. The conditioned medium was assayed for elastolytic activity and plasminogen activator activity.

were coated with IgG and complement (EIgGC), the response showed both the early increase in elastase secretion typical of complement receptor-mediated secretion and the sustained increase typical of Fc receptor-mediated secretion.

*EIgG Does Not Stimulate Elevated Elastase and Plasminogen Activator Secretion by Inflammatory Macrophages Further.* Intraperitoneal injection of certain inflammatory stimuli induces high rates of secretion of neutral proteinases by macrophages (13, 16). Thioglycollate-elicited macrophages, which have high rates of

secretion of elastase and plasminogen activator, showed little stimulation of proteinase secretion after incubation with EIgG (Table IV). Periodate-elicited macrophages, which secreted elastase at high rates and plasminogen activator at low rates, showed little stimulation of elastase but appreciable stimulation of plasminogen activator in response to EIgG. Thus, the magnitude of the stimulation of proteinase secretion by EIgG was greatest when the baseline secretion was lowest.

### Discussion

In this report we demonstrated that the secretion rate of neutral proteinases is modulated by Fc and C3 receptors. EIgG, which are bound and ingested by macrophages via Fc receptors, stimulated secretion of both elastase and plasminogen activator from resident macrophages. Stimulation was dependent on the number of IgG molecules bound per macrophage and was seen at  $>6.2 \times 10^3$  molecules of IgG/erythrocyte, which is equivalent to a density of IgG on erythrocyte surfaces of  $>240$  molecules IgG/ $\mu\text{m}^2$ . This corresponded to a total of 0.99 nM IgG in the medium during incubation ( $8.2 \times 10^{-13}$  molecules of IgG/well containing 0.5 ml of medium), which is in agreement with the reported concentration range of soluble immune complexes (2–63 nM) that stimulates synthesis of complement component C2 (27) and release of lysosomal enzymes (28, 29) and superoxide anion (30) by macrophages. Although Fc fragments have conformations resembling that of the Fc portion of IgG in immune complexes, we did not observe stimulation of elastase secretion by  $\leq 1 \mu\text{M}$  rabbit Fc fragments. Others have reported that Fc fragments of human IgG stimulated collagenase and prostaglandin  $\text{E}_2$  secretion by resident mouse peritoneal macrophages at 0.2–1  $\mu\text{M}$  (31). This apparent discrepancy may be due to differences in recognition of Fc fragments from the two species (human vs. rabbit) or different regulation of the two proteinases (collagenase vs. elastase).

Optimal stimulation of elastase required continuous triggering of receptors for 24 h. Why this was so is not clear. It does, however, suggest that some intermediate or second message must accumulate or be exhausted before secretion is modulated. Similar phenomena have been observed for other receptor-mediated events. For example, cells incubated with epidermal growth factor require 5–6 h of continuous exposure before an irreversible commitment to DNA synthesis occurs, although receptors are down-regulated within 1 h (6). It is interesting to note that after uptake of IgG-coated erythrocyte ghosts, the Fc receptor is also down-regulated for at least 48 h (32). Because macrophage elastase is continuously secreted and has a small intracellular compartment (13), it is likely that stimulation of elastase secretion requires an elevated rate of protein synthesis. In contrast, there is significant cell-associated plasminogen activator, some of which is membrane associated (18, 19). The stimulation of plasminogen activator activity, as detected by the intact cell assay, did not require as long an exposure to ligand as elastase. This phenomenon may be specific to the membrane-bound forms of plasminogen activator (18, 19). The stimulation of elastase by EIgMC was rapid, seen as early as 2 h, and transient, suggesting that stimulation by EIgMC may be due to the temporary accelerated release of elastase from small intracellular stores. Stimulation by EIgMC was not sustained,

probably because macrophages cleave complement fragments, and EIgMC and macrophages do not interact after the cleavage.

It has been suggested that the stimulation of neutral proteinases by phagocytosis may occur because of accumulation of nondigestible particles in secondary lysosomes (11–13), by stimulation of hexose monophosphate shunt (14, 34), or by stimulation of prostaglandin synthesis, which results in elevated cAMP production (35, 36). It is possible that uptake of EIgG and the subsequent flux of EIgG into the secondary lysosomes could affect the function of macrophages; although EIgG are degradable in macrophage lysosomes, the degradation is not immediate. However, the stimulation of neutral proteinases seems to be mediated by the triggering of Fc receptors, because cytochalasin B, which blocked ingestion but not binding of EIgG, did not inhibit the stimulation of neutral proteinases; EIgMC, which are bound but not ingested by resident mouse macrophages via the complement receptor, stimulated the secretion of elastase.

It is known that triggering of Fc receptors rapidly stimulates the respiratory burst (30) and prostaglandin production (37, 38); therefore, these may be the intermediates for increased neutral proteinase secretion. Prostaglandin E may not be responsible for the stimulation, however, because it has also been reported to be inhibitory (39); in addition, IgG<sub>2b</sub>/IgG<sub>1</sub> receptor, but not IgG<sub>2a</sub> receptor, is associated with phospholipase activity and stimulates prostaglandin synthesis (40), whereas both receptors stimulated neutral proteinase secretion in the present study. The respiratory burst may be responsible for the stimulation because 12-O-tetradecanoyl-phorbol-13-acetate stimulates the respiratory burst and also stimulates elastase and plasminogen activator in mouse macrophages (41, 42). Alternatively, ion fluxes may be responsible for the stimulation because the IgG<sub>2b</sub>/IgG<sub>1</sub> Fc receptor has been shown to be an Na<sup>+</sup>/K<sup>+</sup> channel (43).

Our studies on the modulation of proteolytic enzyme secretion in response to binding of EIgG indicate that resident and stimulated macrophages respond differently. Generally, in culture, agents that increase secretion are effective only when the initial secretion rates are low, and agents that decrease secretion are effective only when the initial secretion rates are high. This is in agreement with studies on the modulation of secretion by latex, zymosan, maleylated proteins,  $\alpha_2$ -macroglobulin, and colony-stimulating factor (11, 14, 44, 45), which have indicated that the magnitude of the response is dependent on the state of activation of the macrophages. However, the regulation of secretion is not simple; macrophages with high rates of secretion that are unresponsive to stimulation by these agents are apparently not at maximal secretion rates because they can be stimulated further by other agents (e.g., colchicine and colony-stimulating factor) (45, 46). Also, stimulation of macrophages by endotoxin *in vivo*, which may not result in high secretion by itself, increases the responsiveness of macrophages to modulation by phagocytosis of latex (11).

Macrophages may autoregulate their secretory response to phagocytosis. Elastase secreted by macrophages cleaves IgG into F(ab')<sub>2</sub> and Fc' fragments that do not bind to the Fc receptor (47). Thus, once secretion of elastase is maximal, the enzyme may prevent continued stimulation of Fc receptor-mediated pleiotypic responses.

Proteinases are only a few of the secretion products of macrophages. At

present, these enzymes can be quantified only by their activity, a method that has limitations. For example, because the activity of neutral proteinases may be regulated by inhibitors (15–18), the observed enzyme activity may be due to the regulation of inhibitors. Whether some effects of triggering of the phagocytic receptors of macrophages may result from altered synthesis of proteinase by macrophages remains to be determined.

### Summary

We have determined that the interaction of IgG-coated erythrocytes (EIgG) and complement-coated erythrocytes (EIgMC) with macrophage Fc and complement receptors, respectively, modulates the secretion of the neutral proteinases, elastase, and plasminogen activator. EIgG binding and ingestion stimulated secretion of elastase and plasminogen activator  $\leq 6$ -fold and 20-fold, respectively, over the 3 d following treatment. Stimulation was dependent on the IgG titer bound to each erythrocyte and was detectable at  $>6.2 \times 10^3$  molecules IgG/erythrocyte (total 0.99 nM IgG in the culture). Cytochalasin B did not inhibit stimulation, indicating that the ingestion of ligands was not necessary. Binding of EIgG to the three subclass-specific Fc receptors (IgG<sub>2a</sub>, IgG<sub>2b</sub>/IgG<sub>1</sub>, IgG<sub>3</sub>) was effective. Stimulation of elastase secretion required continued exposure of ligands to cells for up to 24 h, whereas production of plasminogen activator, which has plasma membrane-bound forms as well as secreted forms, was stimulated by exposure for 2 h. The stimulated production of elastase and plasminogen activator by triggering Fc receptors was seen only when the initial secretion rates were low. Periodate- or thioglycollate-elicited macrophages, which have high rates of proteinase secretion, were not stimulated further. EIgMC, which are bound but not ingested by resident macrophages, stimulated elastase secretion transiently, and the rate of secretion returned to the control level by 24 h. Therefore, the mode of stimulation of neutral proteinase secretion by complement receptor differed from that of Fc receptor; stimulation by complement receptor possibly involves a limited release of enzyme from intracellular stores, rather than stimulating accelerated synthesis of enzyme. Erythrocytes coated with both complement and IgG showed both the transient increase in elastase typical of complement-mediated secretion and the sustained increase typical of Fc receptor-mediated secretion. These results suggest that macrophage Fc and complement receptors regulate secretion of proteinases by receptor-specific mechanisms.

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